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Lichenostigma epirupestre, a new lichenicolous species on *Pertusaria* from Spain

Sergio Pérez-Ortega[1] & Vicent Calatayud[2]

[1]*sperezortega@farm.ucm.es*
Departamento de Biología Vegetal II, Facultad de Farmacia
Universidad Complutense de Madrid
Plaza Ramón y Cajal, Ciudad Universitaria, ES-28040 Madrid, SPAIN

[2]*Fundación CEAM*
C/ Charles R. Darwin 14, Parc Tecnològic, E–46980 Paterna, València, Spain

Abstract—The new species *Lichenostigma epirupestre* is described from three localities in Central Spain, growing on *Pertusaria pertusa* var. *rupestris*. Remarks on its taxonomy and closely related species are made and a key to the species of *Lichenostigma* subgenus *Lichenostigma* is also included.

Key words—Iberian Peninsula, lichenicolous fungi, lichens

Introduction

The genus *Lichenostigma* was introduced for *L. maureri* Hafellner (Hafellner 1982) and, since then, a total of 21 species (after www.indexfungorum.org) have been referred to it. Two subgenera are recognized: *Lichenostigma* and *Lichenogramma* (Navarro-Rosinés & Hafellner 1996, Calatayud et al. 2002). Species belonging to subgenus *Lichenostigma* are characterized by having cushion-like ascomata and by the absence of visible vegetative hyphae or strands connecting several ascomata on the surface of the thallus. Taxa included in subgenus *Lichenogramma* show rounded or elongate ascomata that are interconnected by brown hyphae or pluricellular strands. While most species can easily be referred to one of these two subgenera, a few species show some intermediate features. This is the case of species in which the rounded shape of the ascomata is typical of subgenus *Lichenostigma*, but developing immersed (not visible on the host thallus) brown vegetative hyphae, or others that only occasionally develop superficial hyphal strands but only occasionally (Calatayud & Barreno 2003, Halıcı & Hawksworth 2007). The study of some *Lichenostigma* species is difficult due to their small size, morphological variability, high frequency of sterile material and, in some taxa, their diffuse

limits with regard to the species included in the close genus *Lichenothelia* (Calatayud et al. 2002). Recently, the concept of *Lichenostigma* has been enlarged to include also a species with macroconidia (Ihlen 2004), a feature so far considered characteristic of *Lichenothelia* (Henssen 1987). It is expected that molecular studies will contribute to provide relevant information to allow a better delimitation of *Lichenostigma*, and to clarify the relationships among species as well as increase our knowledge of the possible subgroup entities.

During the study of the lichen flora of Tiermes, this being a 3500 years old settlement in the province of Soria (Spain) including a celt-iberic city carved in sandstone, a new species of *Lichenostigma* was collected growing on *Pertusaria pertusa* var. *rupestris*. Further material was later examined from two other localities in central Spain. In the present paper, this new species is described and compared with other related taxa. An artificial key for species of the *Lichenostigma* subgenus *Lichenostigma* is also provided.

Material and methods

Microscopic characters were studied in hand sections mounted in water, 10% KOH (K), Lugol's iodine solution or lactophenol cotton blue, using a Zeiss Axioskop and a Nikon E200 light microscope. All measurements were made in water. For the ascospore size, the range was calculated after manually rejecting 10% of the highest and 10% of the lowest measured values; extreme values are given within parentheses, and the average in italics. Pictures were taken with a Canon PowerShot G2 digital camera fitted to the microscope. Abbreviations: K = 10–35% potassium hydroxide in water; I = Lugol's iodine solution; K/I = pretreatment with K followed by I.

Lichenostigma epirupestre Pérez-Ortega & Calat., **sp. nov.**

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Fungus lichenicola in Pertusaria pertusa *var.* rupestris *crescens. Hyphae vegetativae immersae brunneae. Asci in ascomatibus immersi, 20–24 × 10–14 µm. Hamathecii filamenta absentia. Ascosporae 2(–3)-cellulares, brunnescentes, 11–14 × 6.6–8 µm.*

Etymology: Named in reference to the host species.

Holotype: Spain. Soria: Montejo de Tiermes, old celtiberic settlement of Tiermes, 41°19'49" N, 3°8'59" W, 1170 m, on *Pertusaria pertusa* var. *rupestris* on sandstone, 11 March 2007, Pérez-Ortega (MAF- 15586 HOLOTYPUS).

Morphology. Ascomata superficial on the host (Figs A & B) not connected to other ascomata with pigmented vegetative hyphae (subgenus *Lichenostigma*); ascomata cushion-like, black, irregularly rounded, branched or of a radiating shape (Fig. B), 80–270 µm in diam. (reaching c. 900 µm when several ascomata converge), 40–95(–110) µm thick, flat to slightly convex in central parts, where the asci are produced; lower part of the ascomata sometimes with pale brown

pigmented vegetative hyphae penetrating downwards in the host thallus, mostly simple, very rarely branched, up to c. 65 µm long, cells rounded to elongate, 5–7 × 4.5–6 µm, with a granulose extracellular pigment (Fig. F); branches of 35–70 × 22–40 µm; ascomata not producing any visible damage to the host thallus. Ascomata in section pseudoparenchymatous (Figs C & E), with irregularly rounded cells (3.5–)4–8(–9) µm in diam., external cells brown, internal cells hyaline to light brown. Hamathecial filaments absent. Asci immersed in cavities in the ascomata, bitunicate, broadly saccate, 20–24 × 10–14 µm, fragile, non-amyloid but with a K/I+ blue sheath, amyloid ring not observed, 4–6-spored (Fig. D). Intercellular spaces with a I+ blue, K/I+ blue gel. Ascospores (Figs G–J) brown, ellipsoid to broadly ellipsoid, verrucose when young (Fig. G), very old ascospores with cracked-like surface (Fig. J); 1–2(–3)-septate, very old ones rarely with transverse septa (submuriform), 11–*12.6*–14(–15) × (6–)6.6–*7.1* –8(–9) µm (*n*= 33); lower cell smaller than upper cell, clearly seen in young spores. Anamorph not seen.

Ecology and distribution. To our knowledge, *L. epirupestre* is a lichenicolous fungus growing exclusively on *Pertusaria pertusa* var. *rupestris,* a saxicolous lichen growing on acidic rocks. It has a mainly superficial growth on the host thallus, not causing any apparent injury to the host. So far, the species is known from three localities in central Spain.

Other specimens examined: ***Lichenostigma epirupestre*: SPAIN**. Castilla y León, Ávila, La Hija de Dios, berrocal, 40° 31.346' N, 4° 58.071' W, 1230 m, 4 Oct 2007, Pérez-Ortega & L.G. Sancho, (Herb. Pérez-Ortega); Madrid, La Cabrera, Sierra de la Cabrera, pico de El Mojón, 30TVL4623, 1240 m, 27 Oct 1985, V.J. Rico 374/4 & M.A. Florido (Herb. Rico). ***Lichenostigma*** sp.1: Castilla-La Mancha, Cuenca, Pico Ranera, 30SXK4408, 1400 m, 18 July 1992, on *Pertusaria* cf. *leucosora*, V. Catalatyud (VAL-Lich). ***Lichenostigma*** sp. 2: Andalucia, Almería, Cabo de Gata, San josé, Playa de monsul, 30SWF7565, 15 m, 16 Sept 2000, V. Calatayud & C. Trescolí (VAL-Lich). ***Lichenostigma rugosum***: Burgos, Fresnillo de las Dueñas, N 41° 38'; W 3° 37', 790 m, on terricolous *Diploschistes diacapsis*, 23 May 2006, Pérez.Ortega (Herb. Pérez-Ortega).

Remarks

Before the inclusion of *L. epirupestre*, 7 species had been referred to *Lichenostigma* subgenus *Lichenostigma* (Calatayud et al. 2004). *L. epirupestre* is the only one to date reported on *Pertusaria*. It was found exclusively on *Pertusaria pertusa* var. *rupestris*, a saxicolous lichen on siliceous rocks. In the course of our studies, we also examined two apparently undescribed *Lichenostigma* species growing on saxicolous *Pertusaria* species. Unfortunately, the material was scarce and always sterile, preventing a formal description. *Lichenostigma* sp. 1, was found growing on *Pertusaria* cf. *leucosora* in Cuenca (central Spain). It forms dense networks of radiating and much branched pluricellular strands over the host thallus up to 1 mm in diam (subgenus *Lichenogramma*). Hyphal strands are

10–35 µm thick, composed of up to 8 cells rows (cells 3–6 µm in diam). Only one ascoma has been observed, ± 60 µm in diam, paraplectenchymatic in section, with cells up to 7 µm in diam. The second taxon, *Lichenostigma* sp. 2, was found on an unidentified sorediate *Pertusaria* growing on volcanic rocks (S Spain). It forms a dense network of pluricellular strands (sections up to 25 µm in diam), with cells 3–5 µm in diam. *Lichenostigma* sp. 2 is apparently very close to *Lichenostigma* sp. 1, which resembles under the dissecting microscope. As *Lichenostigma* sp. 1 forms more widespread networks (up to 1 mm in diam) than *Lichenostigma* sp. 2 (up to 0.5 mm) we are sure if they are distinct or not. Both specimens are clearly separated from *L. epirupestre* by their hyphal strand network growing on the host thallus, typical for the subgenus *Lichenogramma*.

In addition to host specificity, *L. epirupestre* is characterized among species of subgenus *Lichenostigma* by the following combination of characters: ascomata mostly irregular in shape (from rounded to ± radiating), relatively small, scattered to confluent, with its lower part sometimes provided with pale brown vegetative hyphae which penetrate downwards the host thallus, and 1 or 2-septate brown ascospores. Similar brown vegetative hyphae arising from the lower side of ascomata are also known in *L. radicans* Calat. & Barreno (Calatayud & Barreno 2003), a lichenicolous fungus described on vagrant *Aspicilia* species. In this species however, the ascomata are rounded and mostly scattered, and the brown vegetative hyphae are longer (up to 180 µm) than in *L. epirupestre* (up to 70 µm), and may be ramified; in *L. epirupestre* they are simple, short and perpendicular to the lower ascomatal surface. *L. canariense* Etayo & Van den Boom, recently described on *Aspicilia calcarea*, differs mainly by its much smaller ellipsoidal or subglobose ascomata, 25–90 µm in diam., and smaller, smooth ascospores, 8.5–10.5 × 5–6 µm (Boom & Etayo 2006). *L. maureri* has more regularly rounded ascomata, which are smaller (70–120 µm in diam.), has a centrum not reacting with I, and the ascospores are slightly smaller (912 36 µm), with only one septum (Hafellner 1982); it grows on diverse fruticose and foliose lichens. *L. rugosum* G. Thor mainly differs from *L. epirupestre* by its ± rounded ascomata, the absence of brown vegetative hyphae, and distinctly halonate ascospores (Thor 1985); in both species, the old ascospores may show superficial fissures and may have more than one septum. The recently described *L. dimelaenae* Calat. & Hafellner (Calatayud et al. 2004) differs from all these species by often laterally compressed ascomata forming dense groups that are

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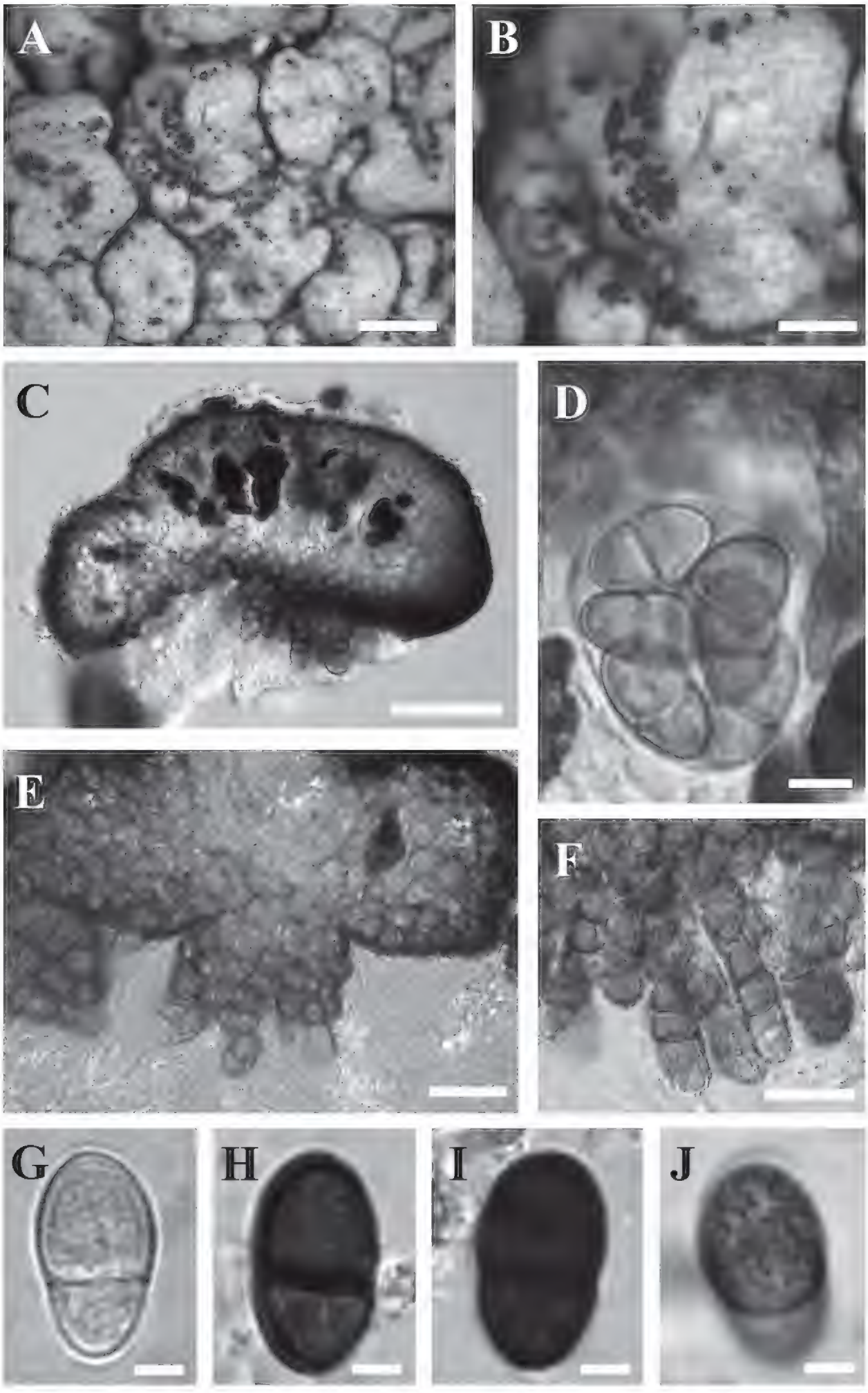
Fig. 1. A & B: Habitus; C: Transverse section of an ascomata; D: Ascus containing 8 ascospores; E: Lower part of an ascoma showing hyphae penetrating in the host thallus; F: Detail of penetrating hyphae in host thallus; G: Young ascospore with granulose surface; H: Mature ascospore; I: Old ascospore showing oblique septa in the lower cell; J: Old ascospore with cracked surface. All microscopical photographs in water, except D in KOH.G, H, I & J made with differential interference contrast.

Scale bars: A: 1 mm; B: 0.5 mm; C: 50 µm; D: 5 µm; E & F: 25 µm; G, H, I, J: 2.5 µm.


A
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inserted laterally on the areoles of the host *Dimelaena oreina*. Other species of the subgenus can be more easily separated from *L. epirupestre* by distinctive characters. *L. hyalosporum* Kalb & Hafellner is the only species with hyaline ascospores, it grows on *Haematomma* (Kalb et al. 1995). *L. triseptatum* Halıcı & D. Hawksw. has been recently described on *Aspicilia*, and its predominantly 3-septate ascospores are considered a diagnostic feature against the other species of this subgenus (Halıcı & Hawksworth 2007). Finally, *L. lecanorae* Calat. & Nav-Ros. grows on *Lecanora farinacea* and has larger ascomata, 100–300 µm wide (Calatayud et al. 2004).

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Key to the species of *Lichenostigma* subgen. *Lichenostigma*[1]

1 Ascospores predominantly 3-septate, 12–16.5 × 6.5–10 µm; on *Aspicilia* ..... ***L. triseptatum***
Ascospores mostly 1(–2)-septate 2

2 Ascospores hyaline; on *Haematomma* ..... ***L. hyalosporum***
Ascospores brown, not on *Haematomma* 3

3 Ascomata 100–300 µm wide and 120–200 µm tall, shape usually irregular; ascospores 10–15 × 6–9 µm; on *Lecanora farinacea* ..... ***L. lecanorae***
Ascomata smaller and shorter; not on *Lecanora* 4

4 Ascomata confluent in dense compact groups, frequently compressed among them and laterally inserted on the host areoles, ascomata 70–140 µm wide and 40–80 µm tall; ascospores 1(–3)-septate, brown, 11–15 × 6.5–11 µm; on *Dimelaena oreina* ..... ***L. dimelaenae***
Ascomata scattered or aggregated but not forming dense groups of compressed ascomata 5

5 Lower part of ascomata frequently producing brown vegetative hyphae that penetrate downwards the host thallus 6
Lower part of ascomata not producing vegetative hyphae or, if present, hyaline and rather indistinct 7

6 Ascomata mostly rounded, flat and scattered; brown vegetative hyphae simple to plurihyphal, up to 180 µm long; wall of inner ascomatal cells brown; ascospores 9–14 × 5–7.5 µm; on vagrant *Aspicilia* ..... ***L. radicans***
Ascomata irregularly rounded to ± radiating in shape, scattered to confluent; brown vegetative hyphae short and simple, perpendicular to the lower ascomatal surface, up to 60 µm long; wall of inner ascomatal cells usually hyaline; ascospores 11–15 × 6–9 µm; on *Pertusaria pertusa var. rupestris* ..... ***L. epirupestre***

7 Ascomata small, 25–90 µm diam, with its basal part composed of a layer of elongated dark brown cells in 1–4 rows; ascospores 8.5–10.5 × 5–6 µm, not ornamented; on *Aspicilia calcarea* ..... ***L. canariense***
Ascomata larger, without elongate basal cells; ascospores larger and ornamented 8

8 Ascomata scattered, 70–120 µm diam; mature ascospores finely ornamented, 9–12 × 3–6 µm; on fruticose epiphytic lichens, especially *Usnea* ..... ***L. maureri***
Ascomata scattered or aggregated, 50–200 µm diam; mature ascospores with deep irregular fissures forming a rough areolate pattern, 10–13 × 5–7 µm; on *Diploschistes* spp. ..... ***L. rugosum***

[1] *Lichenostigma supertegentis* (Ihlen 2004) is not included, as its elongated ascomata and the formation of macroconidia are not typical of the subgenus.